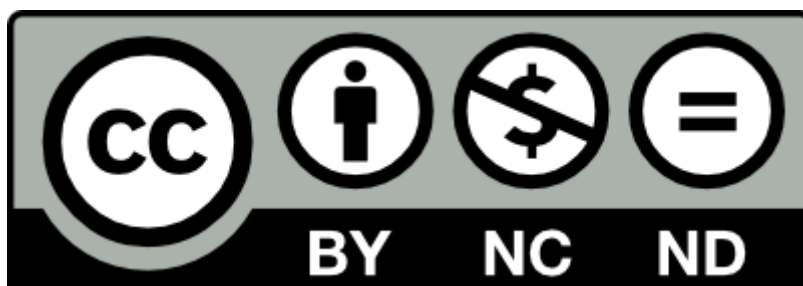


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Abstract: Walnuts are nowadays widely consumed. Since the edible part of walnuts does not account more than 50-60% of their total weight, the total amount of shells produced annually is huge. However, as walnut shells are part of lignocellulosic biomass, they could be valorised via a biorefinery approach in order to extract their diverse constituents. For this reason, the aim of this work was to valorise walnut shells by a biorefinery scheme. The latest involved multiple microwave assisted and conventional hydrothermal treatments for the subsequent valorisation of oligosaccharides. Then, an organosolv delignification of the solid that permitted the maximum oligosaccharide yield was performed, in order to isolate the lignin. Finally, it was treated for cellulose nanocrystal obtaining. The results showed, on the one hand, that the hydrothermal treatments led to xyloligosaccharide-rich liquors (1–17 g/L). On the other hand, the organosolv delignification resulted into the extraction of a highly pure lignin (93.6%) and a weight average molecular weight of 7,000 Da. Moreover, the solid from the delignification treatment was suitable for a successful nanocrystal production. The extracted fractions could be employed in many applications and could be considered renewable precursors for new materials and chemicals. Hence, the proposed biorefinery scheme would allow an integral valorisation of currently undervalued walnut shells.

Keywords: biomass, biorefinery, hydrothermal treatments, lignin, cellulose nanocrystals



**Hydrothermal treatments of walnut shells: a potential pretreatment for
subsequent product obtaining**

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Abstract

Walnuts are nowadays widely consumed. Since the edible part of walnuts does not account more than 50-60% of their total weight, the total amount of shells produced annually is huge. However, as walnut shells are part of lignocellulosic biomass, they could be valorised via a biorefinery approach in order to extract their diverse constituents. For this reason, the aim of this work was to valorise walnut shells by a biorefinery scheme. The latest involved multiple microwave assisted and conventional hydrothermal treatments for the subsequent valorisation of oligosaccharides. Then, an organosolv delignification of the solid that permitted the maximum oligosaccharide yield was performed, in order to isolate the lignin. Finally, it was treated for cellulose nanocrystal obtaining. The results showed, on the one hand, that the hydrothermal treatments led to xyloligosaccharide-rich liquors (1–17 g/L). On the other hand, the organosolv delignification resulted into the extraction of a highly pure lignin (93.6%) and a weight average molecular weight of 7,000 Da. Moreover, the solid from the delignification treatment was suitable for a successful nanocrystal production. The extracted fractions could be employed in many applications and could be considered renewable precursors for new materials and chemicals. Hence, the proposed biorefinery scheme would allow an integral valorisation of currently undervalued walnut shells.

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1. Introduction

The increase of the demand for energy, the growth of greenhouse gas emissions and the depletion of fossil resources have recently led to many social and environmental concerns (Dragone et al., 2020; Ubando et al., 2020). Driven by this situation and due to the current consumerist society, circular economy has gained great attention in the last years. This is a concept of sustainability that limits the use of virgin resources and enhances their recovery and recycling (Ubando et al., 2020). Circular economy also promotes a change from fossil to renewable resources and green processes for energy and material obtaining. In this context, biomass has emerged as an essential renewable source to overcome this situation, especially due to its huge availability and abundance (de Hoyos-Martínez et al., 2018; Ubando et al., 2020). Biorefineries are considered to be a promising way for the conversion of biomass into a variety of products which, at the same time, positively contribute to a circular economy (Dragone et al., 2020). Therefore, biomass and biorefineries are exponentially gaining in popularity.

Among the several types of biomass, the lignocellulosic one almost accounts the 70% of the total plant biomass (de Hoyos-Martínez et al., 2018). This biomass is mainly composed of cellulose, hemicelluloses, lignin and tannins (Morales et al., 2018), which can be extracted and further used for the creation of new chemicals and materials. Lignocellulosic biomass is usually constituted by wastes coming from agricultural and forestry practices, everyday life and alimentary industry. These wastes are generally undervalued, but can be exploited as an inexpensive and renewable source of many bio-compounds. Moreover, the utilization of this biomass eliminates the food versus fuel competition faced by energy production from food crops and the land requirements for growing new plants (Vu et al., 2020). Thus, the employment of agro-alimentary residues as feedstock for biorefineries would contribute both to the valorisation of waste and to the generation of added-value compounds (Gullón et al., 2018).

Tree nuts are nowadays consumed worldwide. Walnut trees (*Juglans regia L.*), for instance, are spread on most of the countries over the northern hemisphere (de Sebastián Palomares, 2008). Together with hazelnuts, walnuts are highly demanded due to their multiple industrial

applications such as oils, liquors, chocolates, nougats and other confectionery specialties, as well as for their direct consumption. In the E.U., due to the high consumers' walnut demand in the last decade, many countries have incremented the harvesting area for this crop (de Sebastián Palomares, 2008). In fact, it is estimated that in Spain each person consumes 0.3 kg of walnut kernel per year (INC-International Nut & Dried Fruit, 2018), just after the Netherlands (0.48 kg/year per capita) and France (0.33 kg/year per capita). However, the edible part of walnuts does not account more than 50-60% of the total weight of the fruit (Hemmati et al., 2018; Orue et al., 2019), which leads to the production of huge amounts of undervalued shells. Recently, they have started to be employed in textile industry as natural dyes (Ali and Nishkam, 2016; Doğan-Sağlamtimur et al., 2017; Mirjalili and Karimi, 2013; Ternua and Archroma, 2019). However, walnut shells are part of the lignocellulosic biomass and could be greater valorised via a biorefinery approach in order to employ this residue as a starting feedstock for the synthesis of new added-value products.

Till date, walnut shells have not been considered as valuable feedstock for any process, but considering their composition (> 35% lignin, \approx 20% cellulose, \approx 30% hemicelluloses and < 15% others) from the biorefinery perspective, they could be a potential source of the aforementioned biopolymers (Ahorsu et al., 2019; de Caprariis et al., 2017; Tan et al., 2019; Wartelle and Marshall, 2000). Hemicelluloses are heteropolymers that can be constituted of xylan, glucuronoxylan, arabinoxylan, glucomannan, xyloglucan and uronic acid, whose content relies on the type of biomass (Ahorsu et al., 2019). Walnut shells, for instance, are known to be rich in xylan (Ahorsu et al., 2019), which can be converted into xylooligosaccharides (XOS) after subjecting the feedstock to an autohydrolysis process (Gullón et al., 2018). XOS can be employed as prebiotic food ingredients, meaning that apart from being beneficial for human gastrointestinal activity, they can promote other effects such as immunological, anti-inflammatory, anticancer and antiallergenic actions (Gullón et al., 2018).

Although many treatments have been studied for biomass fractionation (i.e. enzymatic hydrolysis), hydrothermal treatments such as autohydrolysis have demonstrated to be effective

due to the lack of additional chemicals required, their low equipment corrosion, their simplicity and low-costly and their environmentally friendliness (Ahorsu et al., 2019). Hydrothermal treatments involve the use of liquid water over its boiling point (subcritical, if temperatures are in the range of 100–374 °C, or supercritical above 374 °C and with a pressure of 22.1 MPa), which enables the solubility of small organic compounds at high temperatures due to its lower dielectric constant at these conditions (Torres-Mayanga et al., 2019). These treatments can sometimes lead to the scission of lignin bondages and, therefore, its conversion into low-molecular weight phenolic compounds (Cao et al., 2018). However, these treatments are better known for their high capacity of solubilisation of hemicelluloses, and, at the same time, the production of a solid phase rich in cellulose and lignin, which can be further treated for the extraction of these components (Dávila et al., 2016). The most influential parameter in this process is the way of supplying energy to the reaction medium. Until now, the typically used heating method has been conventional heating. However, the heat transfer rate is relatively low in this case, so intensification techniques such as microwaves have started to be used (Ahorsu et al., 2019). Microwave heating presents several advantages amongst which its rapidity, its biomass flexibility and high final product quality can be highlighted. In addition, this process has also shown to be effective for the extraction of XOS and other compounds from several types of biomass (Ahorsu et al., 2019; Cao et al., 2019). For the extraction of the lignin fraction many treatments have been explored in the last century: organosolv, acid-isolation, alkaline-isolation, Kraft process, ionic liquids, etc. (Dávila et al., 2017). Among them, organosolv delignification has become one of the most promising processes due to the involvement of low boiling points, its simplicity, requirement of non-sulphur formulas and easy recycling possibilities (Erdocia et al., 2014). Organosolv delignification involves the use of organic solvents as reaction medium for biomass conversion. These solvents permit the removal of hemicelluloses and lignin due to the cleavage of the intermolecular connections linked to cellulose, which can also be recovered as solid product with high purity (Jiang et al., 2018).

In this context, the aim of this work was to develop an integrated biorefinery approach of walnut shells using green and emerging processes (see Figure 1) in order to transform an undervalued feedstock into high added-value compounds that could replace non-renewable ones. The scheme proposed involved firstly several hydrothermal treatments of this feedstock, which were carried out via an intensification technique (microwave heating). Then, after selecting the range of temperatures leading to the maximum solubilisation of hemicelluloses, conventionally heated tests were performed. The solid with highest hemicellulosic removal and leading to the highest oligosaccharide production after conventional heating was subjected to an organosolv delignification stage, followed by a bleaching treatment and an acid hydrolysis for the obtaining of lignin and cellulose nanocrystals, respectively. As far as we know, there are no studies on conventional hydrothermal treatment of walnut shells, few studies have been done on the microwave assisted hydrothermal treatments of this feedstock (Ahorsu et al., 2019), and their organosolv delignification process and their use in cellulose nanocrystal production (Hemmati et al., 2018) have not also been deeply explored. Results demonstrate that the proposed biorefinery scheme would enable an integral valorisation of the feedstock, leading to a sustainable co-production of hemicelluloses, lignin and cellulose nanocrystals by means of green biorefinery processes, and contributing to a zero-waste and circular economic model.

2. Materials and Methods

2.1. Materials

Walnut shells (WNS) employed in this work were kindly supplied by a farmer (Zaldibia, Guipúzcoa, Spain). WNS were milled to a particle size between 2 and 1 mm, homogenized in a single lot and stored at room temperature in a dark and dry place until use.

Sulphuric acid (H_2SO_4 , 96%), acetic acid glacial (CH_3COOH) and sodium chlorite (NaClO_2 , 25%) were purchased from PanReac Química SLU. Ethanol ($\text{C}_2\text{H}_5\text{OH}$, for synthesis) was supplied by Scharlab S.L. Toluene (C_7H_8 , certified AR for analysis) was purchased from Fisher Scientific S.L. All reagents were employed as supplied.

2.2. Characterization of the WNS

From the lot of WNS, several aliquots were analyzed according to TAPPI standards for moisture (TAPPI T264-om-88), ashes (TAPPI T211-om-02) and ethanol-toluene extractives (TAPPI T204-cm-97). Klason lignin, acid soluble lignin and carbohydrate content were determined by the protocol TP-510-42618 of the National Renewable Energy Laboratory (NREL), which consisted on quantitative acid hydrolysis (QAH) first with 72% (w/w) H₂SO₄ for 1 h and 30 °C, and then with 4% (w/w) H₂SO₄ for 1 h and 121 °C. The solid phase that was recovered after the QAH was gravimetrically measured and considered as Klason lignin, while the liquid phase was analyzed for the quantification of sugars (glucose, xylose and arabinose), galacturonic and acetic acid by High Performance Liquid Chromatography (HPLC) (Dávila et al., 2017). Acid soluble lignin determined spectrophotometrically (TAPPI UM250-um-83) (Morales et al., 2018). All the analyses were done by triplicate.

2.3. Hydrothermal pretreatments of the WNS

The autohydrolysis treatments of the WNS were carried out in non-isothermal regimen at different temperatures via two different approaches. First, five microwave-assisted (flexiWAVE, Milestone Srl) autohydrolysis were done every 10 °C from 180 to 220 °C. This temperature range was selected according to previous studies (Ahorsu et al., 2019) and prior research carried out in the laboratory. The five treatments had an initial heating ramp of 4.5 min to reach 150 °C, with a fixed power of 400 W. Then, depending on the desired final temperature, the time of the heating varied from 1.5 to 4.5 min. Finally, they were left to cool down to 60 °C. For these experiments, 8 g of sample were mixed with water in a liquid to solid ratio (LSR) of 8 kg/kg (oven dried basis) inside a sealed Teflon vessel with controlled pressure and temperature. The humidity of the sample was taken into account when calculating the total amount of water to be added. Once the vessel was cooled down, the liquid and solid phases were separated by vacuum filtration and small aliquots of the liquid phases were analysed by HPLC while the remaining liquor was stored at

4 °C. The solid phases were dried at room temperature after being washed with distilled water and were afterwards characterized by quantitative acid hydrolysis as explained in Section 2.4..

In addition, the post-hydrolysis of aliquots of each treatment were carried out via acid treatment (4% H₂SO₄ at 121 °C for 30 min) and the reaction products were quantified by HPLC using the same methodology as described in Section 2.2. According to Gullón et al., (2018) (Gullón et al., 2018), the increase in the concentrations of monosaccharides, galacturonic and acetic acid observed during the quantitative post-hydrolysis permits the determination of the concentration of oligomers and their degree of substitution by acetyl and galacturonic groups. Oligosaccharides (OS) were expressed as monosaccharide equivalents. Another aliquot of the obtained liquors was oven-dried at 105 ± 2 °C until constant weight, in order to determine the content of non-volatile compounds (NVC) (Gullón et al., 2018).

For the second hydrothermal approach, WNS were introduced together with water, keeping the previous LSR (8 kg/kg), in a 1.5 L stainless steel 5100 Parr reactor with a 4848 Parr PID temperature controller. The temperatures of these tests were selected according to the results obtained after the microwave treatments, which were 200 and 220°C. After the non-isothermal autohydrolysis, the reactor was cooled down and the liquid and solid phases were again treated as aforementioned.

With the aim of facilitating the comparison of the working conditions, the severity (S₀) of the conventional autohydrolysis treatments was determined. This parameter is defined as the logarithm of the severity factor (R₀) and it considers the effect caused by time and temperature throughout the non-isothermal treatment, including both the heating and cooling period. This parameter is expressed by Eq. 1 (Dávila et al., 2016; Gullón et al., 2018):

$$S_0 = \log R_0 = \log [R_{0Heat} + R_{0Cool}] = \log \left[\int_0^{t_{Max}} e^{\left(\frac{T(t)-T_{Ref}}{\omega}\right)} dt - \int_{t_{Max}}^{t_F} e^{\left(\frac{T'(t)-T_{Ref}}{\omega}\right)} dt \right] \quad (1)$$

where R₀ is the severity factor, t_{Max} is the time (in minutes) needed to achieve the maximum temperature of each autohydrolysis treatment (T_{Max}, °C); t_F is the time (in minutes) required for

the entire heating-cooling cycles; $T(t)$ and $T'(t)$ ($^{\circ}\text{C}$) are the temperature profiles in heating and cooling, respectively, and ω and T_{Ref} are parameters whose values have been reported in the literature ($\omega = 14.75$ $^{\circ}\text{C}$; $T_{\text{Ref}} = 100$ $^{\circ}\text{C}$).

For the microwave assisted experiments, a similar equation (Eq. 2) reported by Ahorsu et al. (2019) and Lopez-Linares et al. (2019) was employed to calculate the severity factor of each process.

$$\text{Severity Factor (SF)} = \log \left[t \times \exp\left(\frac{T-100}{14.75}\right) \right] \quad (2)$$

where t and T are cumulative treatment time (in minutes) and temperature (in $^{\circ}\text{C}$), respectively, and the value of 14.75 is, as in the previous equation, an empirical parameter related to temperature and activation energy (Ahorsu et al., 2019).

2.4. Characterisation of the solid fractions

After air-drying them, aliquots of all the obtained solids were subjected to gravimetric and moisture analyses in order to determine the solid yield and the solubilisation of the raw material. The composition of the solids was analyzed by QAH (TP-510-42618, NREL), as it was done for the raw WNS, in order to determine the Klason lignin and to quantify sugars, galacturonic and acetic acid. All the analyses were done by triplicate.

2.5. Organosolv delignification of the autohydrolysed WNS

After analysing the results obtained from the conventional hydrothermal treatments, the solid from the treatment that led to the maximum oligosaccharide extraction and least degradation product generation was subjected to an organosolv delignification stage. This process was performed at 200 $^{\circ}\text{C}$ during 90 min, using a mixture of ethanol/water (70:30 v/v) and LSR ratio of 1:6 in the abovementioned 1.5 L stainless steel Parr reactor. The operational conditions were selected from a previous study for almond shells (de Hoyos-Martínez et al., 2018) due to the similar composition of both feedstocks. At the end of the treatment, the solid and liquid phases were recovered by vacuum filtration. The solid was firstly washed with an ethanol/water 70:30

(v/v) solution and then several times with distilled water until pH 7, air-dried and weighted to calculate the solid yield. Then, it was characterised as explained in Section 2.4. The black liquor was subjected to lignin precipitation adding 2 volumes of acidified water (H_2SO_4 96% w/w to reach pH 2) so as to decrease the solubility of lignin (de Hoyos-Martínez et al., 2018). The isolated lignin was neutralized with distilled water, air-dried and characterised as described below.

2.5.1. Characterisation of the extracted lignin

The purity of the extracted lignin was determined as reported by Erdocia et al., (2014). Briefly, QAH was performed first with 72% (w/w) H_2SO_4 for 1 h at 30 °C, and then with 12% (w/w) H_2SO_4 for 1 h at 121 °C. The sugar content in the liquor was analyzed by HPLC and the insoluble solid residue obtained was considered as Klason lignin (Erdocia et al., 2014). Further characterization was done according to previous works. These analyses included Pyrolysis-Gas Chromatography/Mass Spectrometry analysis (Py-GC/MS) (Dávila et al., 2017), High Performance Size Exclusion Chromatography (HPSEC) (Morales et al., 2018) and Fourier Transform Infrared Spectroscopy (FTIR) (Dávila et al., 2017).

2.6. Cellulose nanocrystal (CNC) obtaining from the autohydrolysed and delignified WNS

The solid that was previously autohydrolysed and delignified was firstly subjected to a bleaching stage following the method described by Wise et al. (1946) with some modifications. Briefly, 5 g of dry delignified solid were mixed with 1 mL of acetic acid, 5.2 mL sodium chlorite and 160 mL of distilled water in an oil bath at 75 °C under constant magnetic stirring for 2 h. Then, the liquid phase and the bleached pulp were separated by vacuum filtration and the latter was washed with distilled water until neutral pH. The pulp was left to dry at 50 °C.

1 g of the bleached pulp was subjected to an acid hydrolysis with 15 mL of sulphuric acid (50% wt). The reaction was kept at 55 °C in an ultrasound bath for 70 minutes. Then, the slurry was vacuum filtered and washed with distilled water several times until neutral pH. Finally, the CNC were suspended in water and sonicated in order to keep them stable. The CNC obtaining was confirmed via Atomic Force Microscopy (AFM) (Mujtaba et al., 2017).

3. Results and Discussion

3.1. Characterization of the WNS

Figure 2 shows the chemical composition of WNS used in this work and some other compositions reported by other authors. Glucan content represented 18.75% of the dry weight of WNS, which was similar to the values reported by other authors (Ahorsu et al., 2019; de Caprariis et al., 2017). Hemicelluloses accounted the 27.65% of the total composition, and the molar ratio in terms of xylose: arabinosyl groups: galacturonyl groups was 10:1.15: 2.40. Klason lignin content was 33.25% and acid soluble lignin 3.14%. In other works, higher Klason lignin contents were reported (Ahorsu et al., 2019; de Caprariis et al., 2017; Tan et al., 2019), which could be due to the use of larger particle sizes. Wartelle and Marshall (2000), for instance, accounted 18.6% of lignin in their composition (Wartelle and Marshall, 2000). Other fractions, such as ashes (2.45%) and extractives (2.5%), were present at similar concentrations as in other studies (Ahorsu et al., 2019; de Caprariis et al., 2017; Tan et al., 2019; Wartelle and Marshall, 2000). The variation ranges between the bibliographic data shown in Figure 2 could be justified by the differences in the nut varieties used (influenced by factors such as plant genetics, growth environment and processing conditions), and the employed characterization methods (Morales et al., 2018). It was observed that Ahorsu et al. (2019) and Tan et al. (2019) performed a QAH for determining the Klason lignin, cellulose and hemicellulosic content, whereas Caprariis et al. (2017) followed the TAPPI standards and Phyllis Database, and Wartelle and Marshall (2000) got their results from an external laboratory.

3.2. Hydrothermal pretreatments of the WNS

During the autohydrolysis process, the autoionization process of water and the hydrolytic depolymerization of xylan backbone which leads to the generation of acetic acid, increase the acidity of the reaction medium (Surek and Buyukkileci, 2017). Therefore, hydronium ions and acetic acid act as catalysts on the removal of xylan from the lignocellulosic feedstock and enable its degradation into high or low molecular weight XOS and xylose (Surek and Buyukkileci, 2017). Moreover, when microwaves are involved in this process, the ions are instantaneously

superheated and, therefore, the obtained energy transfer is more efficient as temperature increases (Lopez-Linares et al., 2019). On the basis of the chemical composition of WNS, they were hydrothermally treated via two different approaches in order to remove their hemicellulosic fraction, from which oligosaccharides could be obtained. With the aim of studying the maximum hemicelluloses solubilisation, several microwave-assisted experiments of aqueous processing were carried out. The obtained results have been discussed in the next sections.

3.2.1. Effect of the microwave-assisted autohydrolysis conditions on WNS composition and on hydrolysate composition

To assess the suitability of the autohydrolysis treatment for hemicelluloses elimination, several temperatures were tested in the range 180-220 °C (M1-M5) under non-isothermal conditions. Table 1 shows the reaction conditions, the solubilisation of raw WNS and the chemical composition of the spent solids after each microwave-assisted autohydrolysis (MAA) experiment. As it can be seen, the solubilisation was clearly influenced by the increase in temperature and residence time, which was directly related to the severity of the process, and mainly affected non-structural and labile fractions of biomass such as extractives and hemicelluloses (López-Linares et al., 2019). The values for solid solubilisation ranged from 3.84-34.44%, corresponding to the lowest (3.74, experiment M1) and highest severity factors (5.02, experiment M5), respectively. The most significant increase was observed when temperature rose from 210 to 220 °C (13.98% more solubilised solid). In fact, the effect of temperature on the solubilisation was doubly noticeable from 200-220 °C (20.12% more solubilised solid) than from 180-200 °C (10.48% more solubilised solid). The estimated values for severity were aligned with the ones reported by Ahorsu et al. (2019) for WNS and slightly higher than those published by Lopez-Linares et al. (2019) for brewer's spent grain. However, the latest observed lower solid recoveries, which could be either related to the composition of the employed biomass or to the operational conditions. It is known that at harsher reaction conditions, more acetyl groups from hemicelluloses are solubilised, which leads to a drop in the pH of the medium and an increase on its severity (Lopez-Linares et al., 2019). Therefore, the recovery of the final solid is impoverished.

As shown in Table 1, glucan (13.00-18.49%) and Klason lignin (34.88-41.73%) content increased steadily from the experiments M1-M4, but a more notable increment was seen on the glucan content of the solid after the experiment M5 (27.33%), which had a 45.81% of lignin. So, the spent solid got enriched in glucan and lignin, which are the least susceptible fractions to autohydrolysis (Gullón et al., 2018). In the case of glucan, however, a notable decrease was seen at the lowest temperature. A similar behaviour was reported by Dávila et al. (2016), who saw that in the range 180-200 °C less glucan was retained in the solid phase than in conventional treatments at higher temperatures and severities. Lopez-Linares et al. (2019) also observed that at their microwave assisted treatments below 150 °C no glucan enrichment happened, which they related to the limited hemicelluloses solubilisation under the employed conditions. Xylan, galacturonic acid and acetyl group contents decreased continuously from M1-M5, since these components are highly susceptible to hydrolytic degradation (Gullón et al., 2018). Xylan content got firstly decreased with respect to the initial xylan content, but for experiments M2, M3 and M4 this content was higher than for the rest of the experiments, meaning that other hemicelluloses were being solubilised. However, a drastic drop on this fraction was seen for experiment M5, meaning that the severity (residence time and temperature) of this experiment had important effect on the solid. Ahorsu et al., (2019) reported that these two factors had a significant effect on xylose yield (Ahorsu et al., 2019). The spent solid from M5 lacked on xylan and had low galacturonic acid (1.08%) and acetyl group (0.38%) contents. Arabinan was not detected in any of the spent solids.

Table 2 shows the effects caused by the hydrothermal processing on the composition of the liquors. Under adequate conditions, these treatments enhance reactions that lead to the solubilisation of hemicelluloses and other fractions producing both non-volatile (NVC) and volatile compounds (VC) (Dávila et al., 2016). As the temperature of the treatments rose, the substrate conversion into NVC also increased from 3.82-24.37% (oven dried weight), reaching the maximum after the experiment M5. The substrate conversion into VC ranged from 0-0.74% (o.d. weight), which meant that few degradation products from glucose and xylose such as 5-Hydroxymethylfurfural (HMF) and furfural were generated (Ahorsu et al., 2019).

Among the obtained oligosaccharides, the xylooligosaccharides (XOS) were the most abundant ones. The highest xylan to XOS conversion (62.42%, M5) led to a XOS concentration of 14.65 g/L (see Figure 3a), which was higher than the ones reported by other authors for chestnut shells (7.1 g/L), vine shoots (12.2 g/L) and hazelnut shells (10.1 g/L) via conventional hydrothermal treatment (Dávila et al., 2016; Gullón et al., 2018; Surek and Buyukkileci, 2017). Other oligosaccharides were present in the autohydrolysis liquors at lower concentrations. Arabinosyl substituents, for instance, presented the highest conversion into arabinose monosaccharide after M3 treatment (3.44%) and the conversion of acetyl groups linked to oligomers (AcOS) increased substantially from M1-M5 (0.57-58.64%).

Acetic acid was the principal degradation compound present in all the autohydrolysis liquors (see Figure 3b). This compound tends to accumulate in the reaction medium when acetyl groups have been hydrolysed (Gullón et al., 2018). At 180 °C, the concentration of acetic acid was low (0.12 g/L), whereas as the temperature increased, this concentration reached 1.75 g/L. The highest increment on degradation products (acetic acid, furfural and HMF) was observed from 200 °C on, (M3-M5), but still the joint concentration of these products (0.37-2.72 g/L) was lower than the one reported for WNS by Ahorsu et al. (2019) in most of their experiments with similar severity factors. However, this might be related to their longer reaction times, which could have contributed to the production of more degradation compounds.

3.2.2. Effect of the conventional autohydrolysis conditions on WNS composition and on hydrolysate composition

Taking the results from the MAA into account, conventional hydrothermal treatments were carried out in a reactor at 200 (C1) and 220 °C (C2), since it was seen that at least in MAA temperatures below 200 °C did not have significant effect on the solid composition, and that the difference of 10 °C (from 200-210 °C or 210-220 °C) was not very notable either. The results in Table 1 showed that the solubilisation increased greatly in comparison to the ones under microwave-assistance, i.e. at 200 °C a 14.32% of the initial solid got solubilised in the latest while under the conventional method a 35.73% was solubilised, which might be related to the difference

on the heating method and reaction times. In fact, the heating and total reaction times for M3 experiment were of 7 and 28 minutes, respectively, whereas for C1 these times were 54 and 67.5 minutes, subsequently. A similar solubilisation was observed for chestnut shells, hazelnut shells and vine shoots at the same temperature by Gullón et al. (2018), Surek and Buyukkileci (2017) and Dávila et al. (2016). Bichot et al. (2020) also reported higher solubility values using conventional heating compared to the ones obtained by microwave-assisted heating. Although the difference on the obtained Klason lignin content was just of 8.96% between both hydrothermal treatments at 200 °C, the rest of the components (cellulose and hemicelluloses) presented bigger variations. This dissimilarity was slightly more remarkable on the experiments carried out at 220 °C in the case of Klason lignin, which was 9.67% higher on the conventionally-treated solid, but the changes in the remaining fractions were more moderate.

As seen for MAA experiments, the solids were enriched in glucan and lignin together with the hemicelluloses removal. The higher glucan and lignin contents as well as the lack of xylan confirmed the greater severity of the conventional autohydrolysis at 220 °C (4.62) than the one at 200 °C (3.90). Similar severity values were reported by Dávila et al. (2016), who used the same operational conditions and reactor.

Regarding the composition of the obtained liquors (see Table 2), C1 led to a higher conversion of substrate into NVC than C2 (24.37% vs. 9.22%, respectively) and also than M3, which was expected due to the reported higher solubility, as aforementioned. On the contrary, the VC content was five times more elevated for C2 than for C1, which would be directly related to the generation of degradation products due to a higher severity of the process (4.62% Vs. 20.64% for C1 and C2, respectively) (Dávila et al., 2016). The MAA experiments did also led to a significantly lower production of VC.

After C1, monosaccharides such as xylose (3.22 g/L) and arabinose (0.09 g/L) were present in the liquor, and the concentration on xylooligosaccharides (XOS) was also high (16.85 g/L) (see Figure 3a), which was triply higher than the one reported for XOS in M3 (5.88 g/L). This XOS concentration after C1 corresponded to a conversion of xylan into XOS of 71.88%, which was a high yield comparing to the ones reported in literature for the autohydrolysis of many agricultural

byproducts (41.2-61.2%) (Nabarlatz et al., 2007) and chestnut shells (56%) (Gullón et al., 2018), but lower than that reported for vine shoots (83.1%) (Dávila et al., 2016). Moreover, the percentage of acetyl groups belonging to oligosaccharides was elevated (59.98%), which could be attributed to the XOS obtained from acetylated lignocellulosic biomass (Surek and Buyukkileci, 2017). Although the detected xylan to xylose conversion was slightly lower in the case of C2 (3.49% less in C2), no oligosaccharides were detected in this liquor. This means that they were converted into degradation products, and would be in agreement with the results reported by Gullón et al. (2018), who noticed that above 200 °C, the dehydration byproducts of xylose and glucose (i.e. furfural and hydroxymethylfurfural, subsequently) were easily generated. The most abundant degradation compound was, however, as for MAA experiments, acetic acid, although in the microwave-assisted case the produced concentrations were lower. Whereas at 200 °C the acetyl group conversion into acetic acid was of 7.47%, at 220 °C this conversion rose to 100.00%, which led to a concentration of 6.37 g/L (see Figure 3b). Ahorsu et al. (2019) reported similar acetic acid concentrations for lower temperatures after their MAA experiments with WNS. Furfural was also abundant, especially in the case of C2, in which a concentration of 6.61 g/L was detected. The joint concentration of degradation products in this work was higher than that reported for vine shoots at 200 °C and for chestnut shells at 220 °C by other authors (Dávila et al., 2016; Gullón et al., 2018).

Even if hemicelluloses have been less exploited than other fractions from biomass such as cellulose, the interest on obtaining oligosaccharides from hemicelluloses has grown lately due to their applicability in several fields such as healthcare, cosmetics, biopolymers and coatings (Dávila et al., 2016). Nevertheless, it should be noted that the ester bonds binding acetyl groups to the xylan are hydrolysed during autohydrolysis, generating acetic acid. This acid acts as a catalyst in the reaction and it hydrolyses xylan into xylooligosaccharides. It is known, however, that acetic acid is toxic to microorganisms and, hence, its presence should be avoided in prebiotic products from hemicelluloses (Surek and Buyukkileci, 2017). So, taking the latter into account, it could be said that the simplicity and environmentally friendliness of the employed process for

the extraction of hemicelluloses from WNS would enable their usability in all the above mentioned disciplines.

3.3. Organosolv delignification of the autohydrolysed WNS

Results from the conventional hydrothermal treatments of WNS (C1 and C2), suggested that the best temperature for the maximum oligosaccharide extraction and minimum formation of degradation products was 200 °C. Therefore, the spent solid from this treatment was subjected to an organosolv delignification stage in order to study the influence of the hemicellulosic presence on the lignin extraction. To our knowledge, it is one of the first times that an organosolv treatment has been employed for the production of lignin from autohydrolysed WNS. Other treatments such as acidic and alkaline ones have already been employed (Hemmati et al., 2018; Tan et al., 2019), but there is few information about the organosolv ones (Zijlstra et al., 2019). The treatment conditions, as abovementioned, were selected according to a previous study on almond shells (Fernández-Rodríguez et al., 2017), which reported high lignin amounts in their samples, and presented a similar visual appearance to WNS. The composition of the spent solid after the organosolv treatment was 42.54% glucan, 7.57% xylan, 0.92% acetyl groups, 29.28% Klason lignin and almost a 20% of undetermined compounds. The calculated overall lignin yield was 28.7%, which was estimated relating the extracted amount of lignin to the mass of the input biomass, and the relative yield 59.8%, which was assessed taking the extracted lignin and the lignin content in the input biomass into account.

As abovementioned, the spent solid moved from having 28.00% glucan, 6.84% xylan and 48.05% lignin to a 42.54% glucan, 7.57% xylan and 29.28% of lignin. The treatment led to a relative lignin yield of almost 60%, meaning that from the total amount of lignin present in the solid before the treatment the aforementioned percentage was removed (de Hoyos-Martínez et al., 2018). It was observed that the overall yield ($\approx 29\%$), which was calculated relating the extracted amount of lignin to the mass of used biomass (de Hoyos-Martínez et al., 2018), was higher than that reported by Fernández-Rodríguez et al. (2017) for the organosolv delignification of previously autohydrolysed almond shells (12-20%). Moreover, comparing to the overall yield reported for

untreated almond shells (16.75%) (de Hoyos-Martínez et al., 2018), the overall yield achieved in this work was considerably greater.

3.3.1. Characterisation of the extracted lignin

The chemical composition of the extracted lignin was of 93.60% Klason lignin, 0.85% acid soluble lignin, 1.2% glucan, 0.5% hemicelluloses and 3.85% undetected compounds. Therefore, based on the amount of Klason lignin, the purity was high. These results are aligned with the ones reported by Fernández-Rodríguez et al. (2017) for previously autohydrolysed and delignified almond shell and olive tree pruning organosolv lignins.

From the Py-GC/MS analysis, the identification and quantification of the compounds with a higher abundance than 0.4% was carried out (see Table 3). These compounds arose mainly from the lignin moiety, confirming its elevated purity, in spite of presenting some carbohydrate degradation products and fatty acids. The products generated from lignin were classified into four groups depending on their aromatic structure: derived from guaiacol (G), derived from p-hydroxyphenyl (H), derived from syringol (S) and derived from catechol (C) (Chen et al., 2015; Morales et al., 2018). The sum of the identified compounds accounted around an 85% of the detected ones, and the ratio S/G was calculated both by incorporating and without incorporating the C-type compounds as S-derived degradation products. The most abundant aromatic compounds from the pyrolysis included p-cresol (4.4%), guaiacol (11.6%), 4-methylguaiacol (11.5%), 3-methoxycatechol (3.5%), 4-ethylguaiacol (4.6%), 4-vinylguaiacol (4.6%), syringol (10.0%) and 4-methylsyringol (7.8%). Besides, fatty acids were also detected (3.4%), since nuts tend to have many lipophilic extracts (Queirós et al., 2020). From these results, it was concluded that G-type compounds were the most abundant, followed by the S-type, H-type and finally the C-type compounds, which led to a S/G ratio of 0.56 and a (S+C)/G ratio of 0.66. According to Ma et al. (2016), the direct cleavage of the β -O-4 bondages, which have the lowest dissociation energy, lead to a G-type phenolic production. Although Queiros et al. (2019) reported S/G >1 ratio for whole walnut shells, it is known that this ratio can vary significantly based on the pulping or extraction methods employed (Tagami et al., 2019).

From its molecular weight distribution it was estimated that the weight average (M_w) and number average (M_n) molecular weights of this lignin were around 7,000 Da and 1,000 Da. Thus, the polydispersity index was of 6.4, despite the 85% of the sample presenting a molecular weight of around 8,000 Da (see Table 4). These values are in accordance with the ones reported by other authors for organosolv lignins (de Hoyos-Martínez et al., 2018; Erdocia et al., 2014); Domínguez et al., 2018).

The FTIR spectra of this lignin presented the typical bands for organosolv lignin (see Figure 4), which was in agreement with the ones reported by Sequeiros and Labidi (2017) for their organosolv lignins coming from different extraction processes (Sequeiros and Labidi, 2017). Briefly, the band at 3460 cm^{-1} represented the -OH stretching vibration and the ones at 2950 and 2840 cm^{-1} were attributed to the C-H vibration of methyl and methylene groups. The peak around 1710 cm^{-1} was related to the C=O stretch vibration in unconjugated ketones, and the ones at 1600 and 1510 cm^{-1} to the C=C of aromatic skeletal vibration. The band at 1460 cm^{-1} represented the asymmetric deformation of C-H groups, the one around 1425 cm^{-1} the aromatic skeletal vibration and the ones at 1335 and 1220 cm^{-1} the C-O stretching of the syringil and guaiacyl rings, respectively. At 1270 cm^{-1} a shoulder was detected, which was probably related to the impurities such as hemicelluloses (Orue et al., 2019). The peaks around 1125 and 920 cm^{-1} were ascribed to the C-H in plane deformation of syringyl and guaiacyl units, subsequently. The band at 1030 cm^{-1} also represented the hemicellulosic impurities, and the band at 829 was attributed to the β -glucosidic linkages, which were still present due to cellulosic impurities (Sequeiros and Labidi, 2017).

3.4. Cellulose nanocrystals (CNC) obtaining from the autohydrolysed and delignified WNS

The solid that was previously autohydrolysed and delignified was subjected to a bleaching stage and an acid hydrolysis for the production of CNC. The AFM images confirming the CNC obtaining are shown in Figure 5 along with their typical surface height profile. The morphological properties and dimensions of the obtained CNC were estimated from these images. As seen, the CNC were sharp and presented typical rod-like structures with an average length (L) and diameter

(D) of 475.70 ± 90.21 and 44.17 ± 8.46 nm, respectively. This would lead to an average aspect ratio (L/D) of 10.77. According to Figure 5c, the roughness of the analysed surface was not totally homogeneous, but it was seen that the average value was around 9.5 nm, although there was slight variation in the range of 8–11.6 nm. These results were aligned with those reported by other authors for the CNC coming from lignocellulosic biomass (Herrera et al., 2016; Mujtaba et al., 2017; Xiao et al., 2019), although the aspect ratio was more similar to the one reported by Babu Perumal et al. (2018). Comparing to the results published by Hemmati et al. (2018) for WNS CNC, the dimensions obtained in the present study were greater. This could be directly related to the reaction conditions (time, temperature and acid concentration), as demonstrated by these and other authors (Hemmati et al., 2018; Xiao et al., 2019), as well as to the measuring technique.

4. Conclusions

In this study the valorisation of WNS, an abundant waste generated from the agro alimentary sector, was proposed by dint of microwave assisted and conventional hydrothermal treatments, a subsequent organosolv delignification and a bleaching and acid hydrolysis treatment for cellulose nanocrystal production. On the one hand, it was seen that the conventional hydrothermal treatment was more effective for hemicellulosic removal than the one that was microwave-assisted. Nevertheless, it was observed that the xyloligosaccharides concentration on the resulting liquors was similar via both techniques (14.65 g/L after M5 and 16.85 g/L for C1), which would reduce the environmental impact of this process. The solids recovered after all the autohydrolysis processes were enriched in cellulose and lignin. On the other hand, the addition of a delignification stage to the optimal autohydrolysis, led to the obtaining of a cellulose-rich pulp together with a highly pure lignin stream (96.3% pure), achieving a relative yield of 60%. Moreover, the treatment of this pulp permitted a successful production of cellulose nanocrystals (40% conversion). This means that per kilogram of input WNS, around 135 g of XOS, 186 g of lignin and 90 g CNC would be produced. The extracted oligosaccharides could be employed in many fields, lignin would be an interesting renewable feedstock for the synthesis of new materials and chemicals, and the cellulose-rich pulp could be employed as source of nanocrystals, which

could be employed as fillers in many composite materials. Thus, it can be concluded that WNS would be a suitable feedstock for an integral valorisation, contributing to a zero-waste and circular economy.

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Figure list

Figure 1. Flowchart of the biorefinery scheme developed for the overall valorisation of walnut shells.

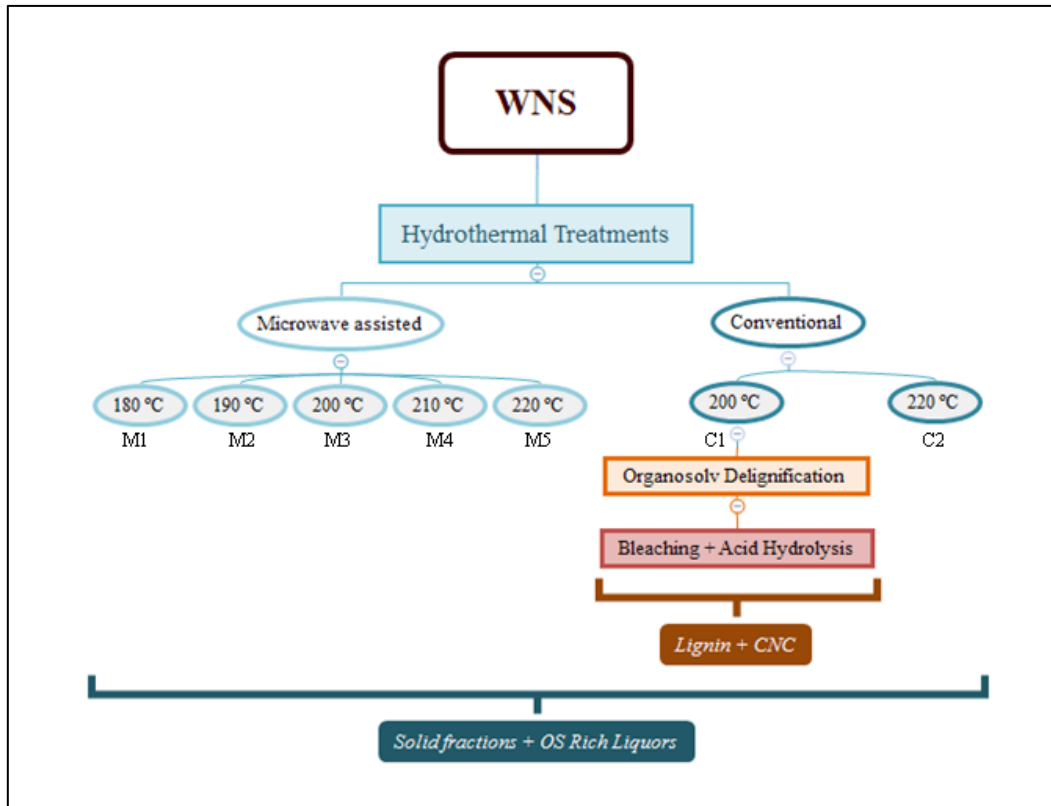


Figure 2. Composition of WNS compared with literature.

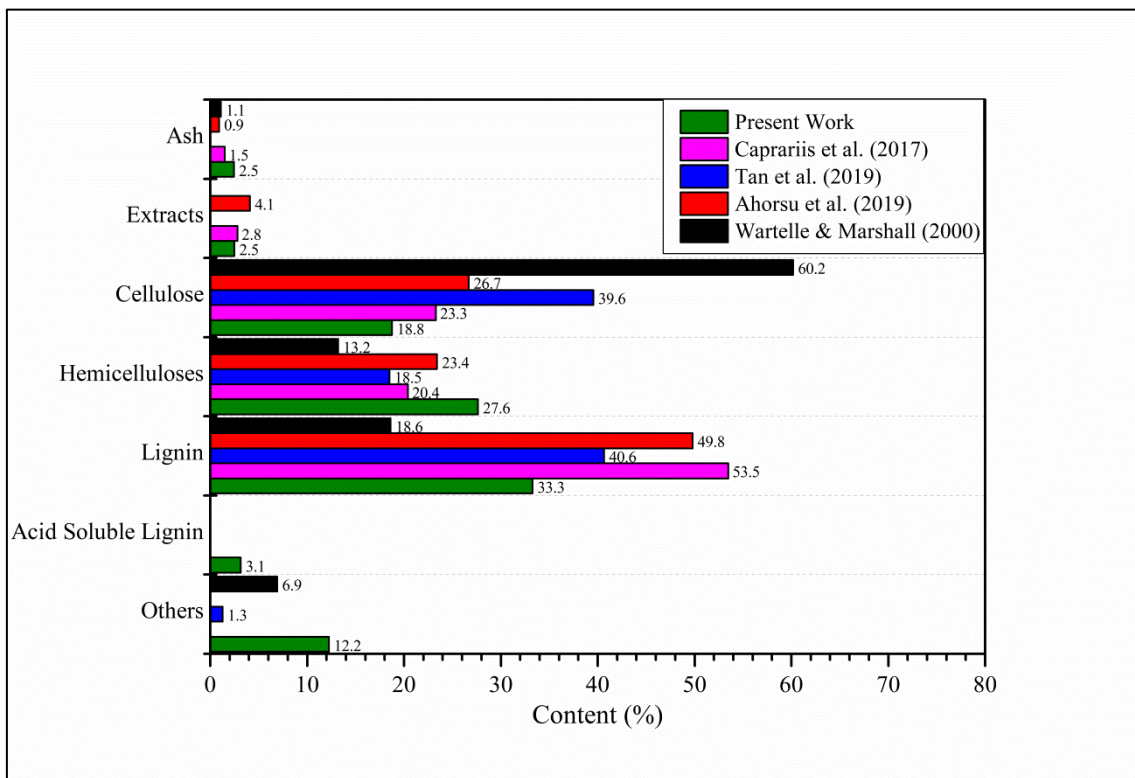


Figure 3. Effect of hydrothermal treatments on the composition of liquors: a) monosaccharides (glucose, xylose and arabinose) and oligosaccharides (GOS, XOS and ArOS); b) degradation compounds (acetic acid, furfural and HMF).

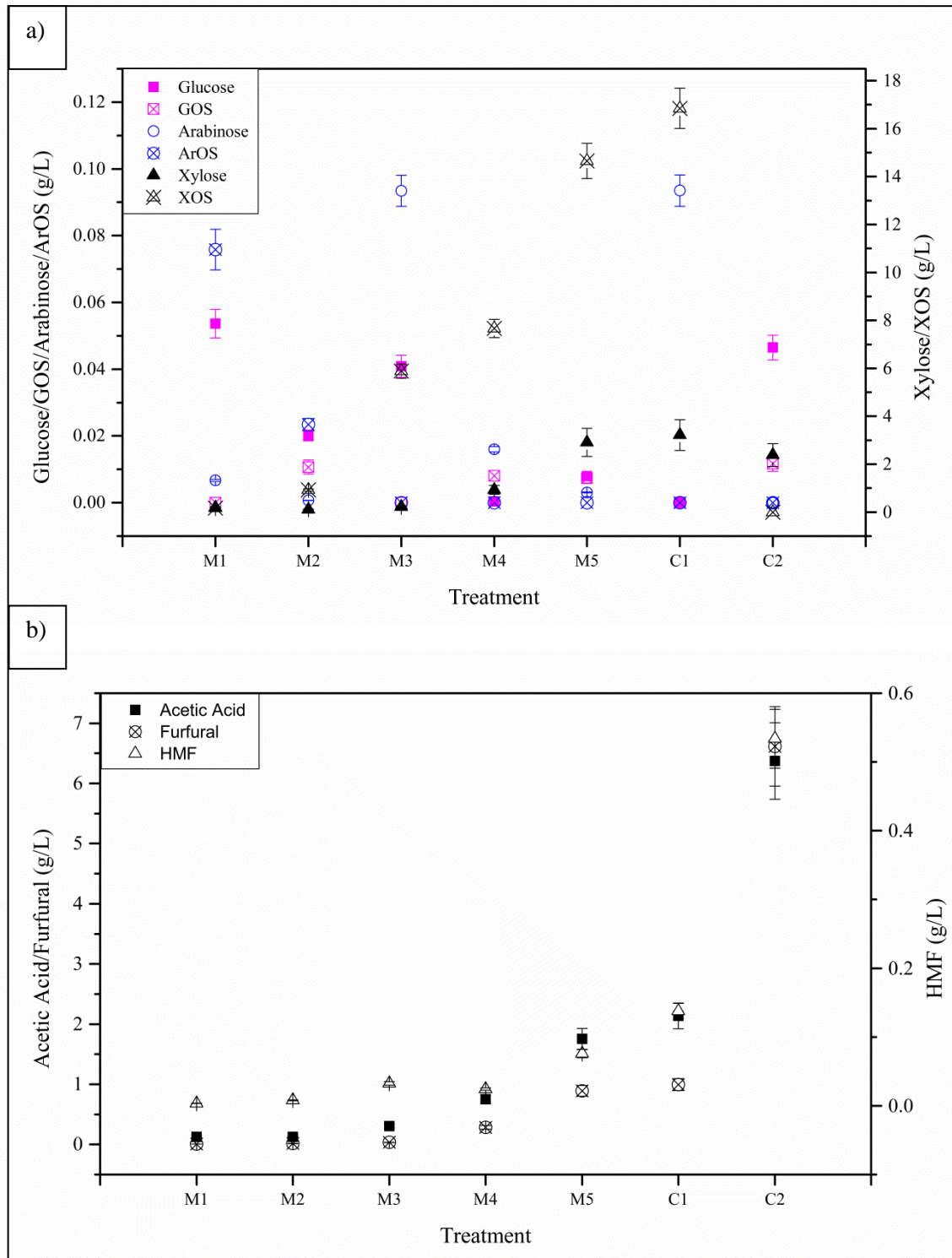


Figure 4. FTIR spectra of the extracted lignin.

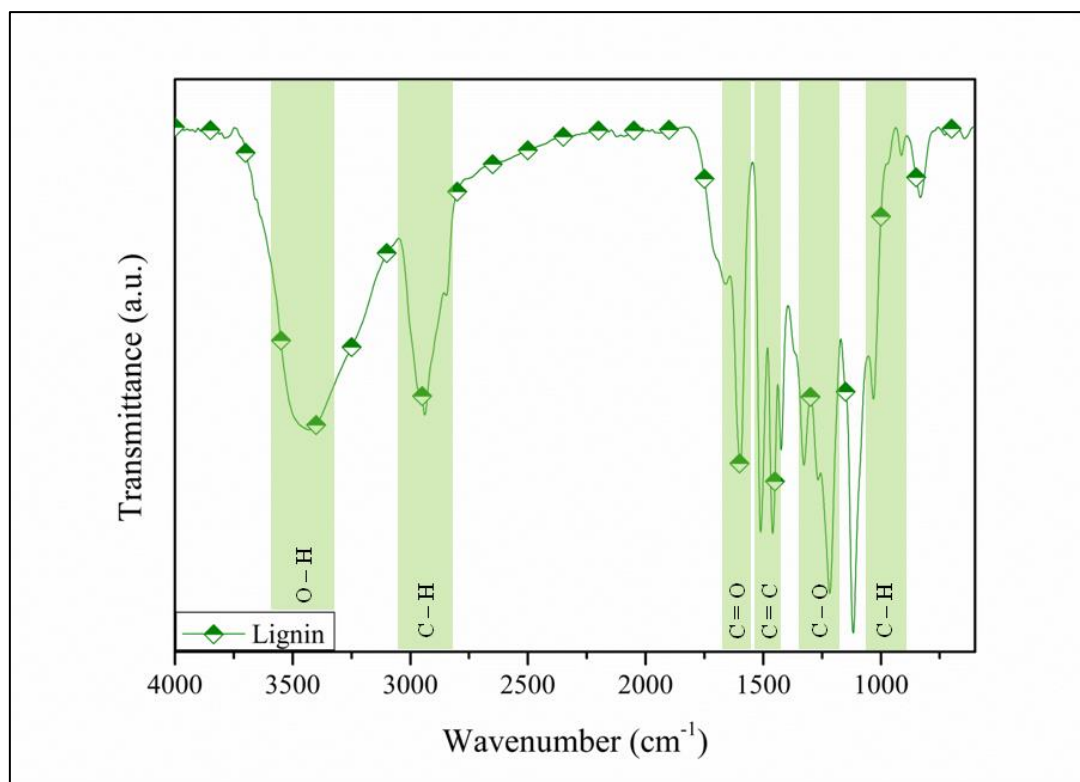
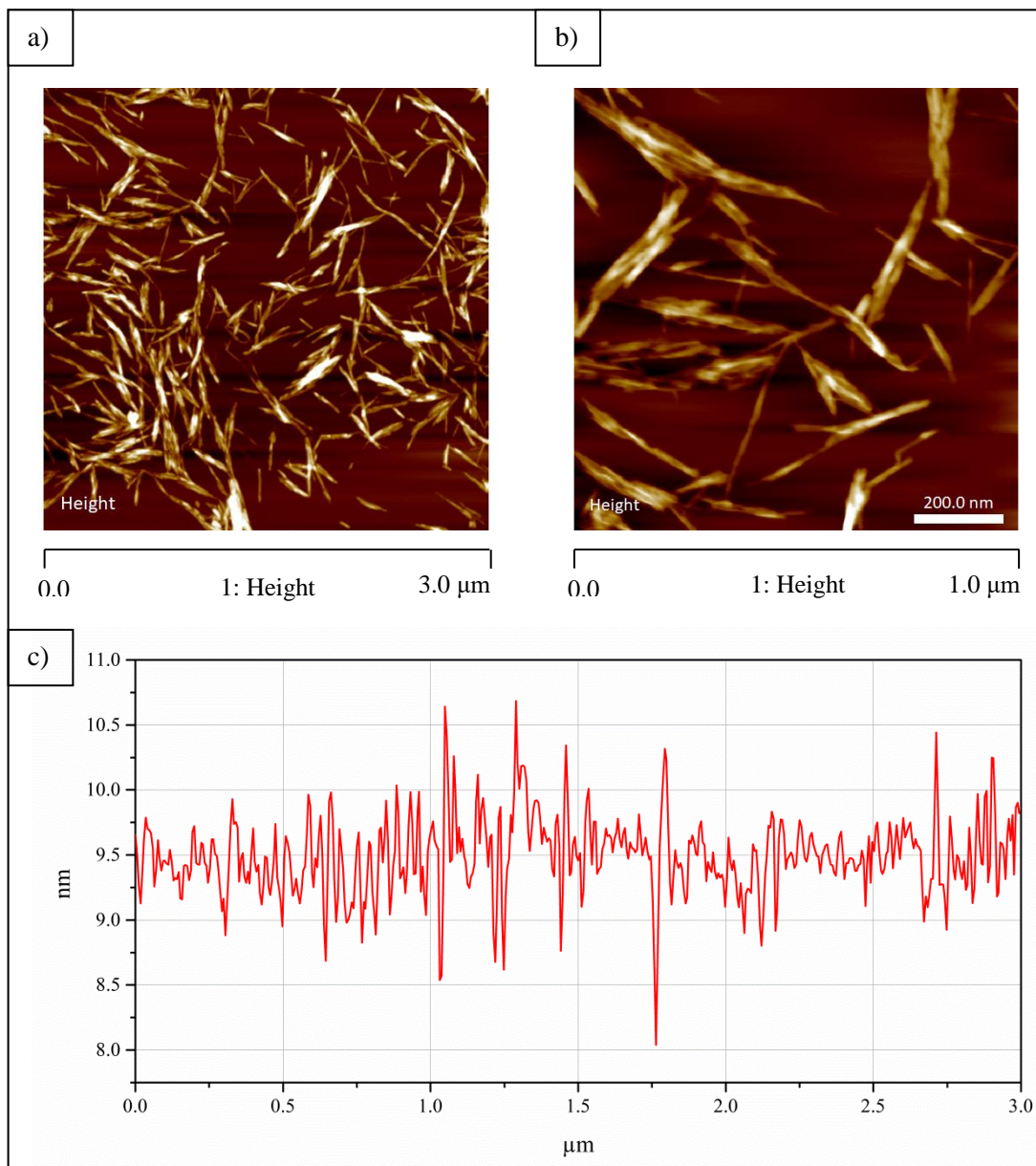


Figure 5. AFM images (a and b) and profile of the height values (c) along the sample in the marked area of 2D AFM images of WNS CNC.



List of Tables

Table 1: Solubilization, severity and chemical composition of the spent solids after MAA experiments and CA treatments.

Treatment		M1	M2	M3	M4	M5	C1	C2
	Time (min)	24	26	28	30.5	33	67.5	100.4
	Temperature (°C)	180	190	200	210	220	200	220
	Severity	3.74	4.06	4.39	4.76	5.02	3.90	4.62
	Max. Power (W)	400	400	400	400	400	-	-
	Solubilization (%)	3.84	6.02	14.32	20.46	34.44	35.73	41.84
	Glucan	13.00	14.85	17.86	18.49	27.33	28.00	32.98
	Xylan	17.90	18.33	19.62	16.60	7.28	6.84	0
	Arabinan	0	0	0	0	0	0	0
Composition (%)	Galacturonic acid	3.27	3.25	3.00	2.85	0.85	1.13	1.08
	Acetyl groups	3.35	3.45	3.73	3.23	1.00	1.19	0.38
	Klason lignin	34.88	35.54	39.09	41.73	45.81	48.05	55.48
	Others (by difference)	27.60	24.58	16.70	17.10	17.73	14.79	10.08

Table 2: Effect of MAA and CA treatments on the liquors composition.

Treatment	M1	M2	M3	M4	M5	C1	C2
Substrate conversion into NVC ^a (% o.d. weight)	3.82	4.07	13.94	14.19	24.37	24.37	9.22
Substrate conversion into VC ^b (% o.d. weight)	0.00	0.14	0.03	0.45	0.73	4.62	20.64
Glucan conversion into Glucose (%)	0.19	0.08	0.17	0.00	0.03	0.00	0.20
Xylan conversion into Xylose (%)	0.76	0.45	0.93	3.88	12.39	13.73	10.24
Arabinosyl substituents conversion into Arabinose (%)	0.22	0.03	3.44	0.59	0.12	3.53	0.00
Acetyl groups conversion into Acetic acid (%)	0.00	0.08	0.01	0.44	0.97	7.47	100.00
Glucan conversion into GOS ^c (%)	0.00	0.04	0.00	0.03	0.03	0.00	0.05
Xylan conversion into XOS ^d (%)	0.52	3.80	24.45	32.09	62.42	71.88	0.00
Arabinosyl groups conversion into ArOS ^e (%)	2.31	0.85	0.00	0.00	0.00	0.00	0.00
Acetyl groups conversion into AcOS ^f (%)	0.57	4.04	26.06	30.62	58.64	59.98	0.00

^aNon-Volatile compounds; ^bVolatile compounds; ^cGlucooligosaccharides; ^dXylooligosaccharides; ^eArabinose substituents of oligosaccharides; ^fAcetyl groups of oligosaccharides

Table 3: Identified Py-GC/MS compounds for the extracted lignins.

RT	Compound	m/z	Lignin	Origin
7.47	Phenol	94/66/65	3.61	H
8.69	o-Cresol	108/107/79	1.55	H
9.03	p-Cresol	107/108/77	4.38	H
9.35	Guaiacol	109/124/81	11.61	G
10.34	Phenol, 3,5-dimethyl-	122/107/121	1.40	H
10.72	Phenol, 4-ethyl-	107/122/77	1.26	H
11.07	3-Methylguaiacol	123/138/77	1.54	G
11.45	4-Methylguaiacol	138/123/95	11.51	G
11.93	Coumaran	120/91/119	0.66	CH ^a
13.37	3-Methoxycatechol	140/125/97	3.45	C
13.90	4-Ethylguaiacol	137/152/15	4.60	G
14.23	4-Methylcatechol	124/123/78	<0.4	C
15.07	4-Vinylguaiacol	135/150/107	4.57	G
16.14	Syringol	154/139/111	10.05	S
16.25	Eugenol	164/103/77	1.53	G
16.34	Phenol, 3,4-dimethoxy-	154/139/111	1.81	S
16.48	Guaiacylpropane	137/166/122	1.27	G
17.27	Vanillin	151/152/81	0.85	G
17.42	cis-Isoeugenol	164/77/149	1.11	G
18.29	4-Methylsyringol	168/153/125	7.75	S
18.98	Acetovanillone	151/166/123	0.87	G
19.63	5-tert-Butylpyrogallol	167/182/139	1.26	H
20.26	Acetoveratrone	165/180/79	1.23	G
20.82	4-Allylsyringol	194/91/119	0.43	S
21.50	cis-4-Allylsyringol	194/91/179	0.42	S
21.60	Syringaldehyde	182/181/111	0.41	S
22.15	trans-4-Allylsyringol	194/91/179	0.81	S
22.58	Acetosyringone	181/196/43	0.82	S
23.02	Homosyringic acid	167/212/168	<0.4	S
23.64	Propiosyringone	181/210/182	<0.4	S
25.00	Fatty Acids		3.35	F ^b
	Others (by the difference)		15.10	
H-type	Total p-hydroxyphenyl derivatives		13.46	
C-type	Total catechol derivatives		3.83	
G-type	Total guaiacyl derivatives		40.69	
S-type	Total syringyl derivatives		22.95	
	Ratio S/G		0.56	
	Ratio (S+C)/G		0.66	

^aCH: carbohydrate derivative compounds; ^bF: Fatty acids

Table 4: HPSEC results for the sequentially extracted lignins.

Lignin Sample	Retention time (min)	Mw (Da)	Percentage (%)	Average Mn (Da)	Average Mw (Da)	Average Mw/Mn
L4	21.56	8,189	86.35	1,115	7,113	6.38
	24.59	387	7.39			
	25.66	236	3.27			
	26.82	173	2.99			